THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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NEW YORK 21, N.Y.

March 1, 1954

Dear Joshua :

Thanks ever so much for the copy of the report. It is quite apparent that things have been moving along on many fronts. I was very pleased with the results of the micro-manipulation experiments which seem to completely remove any notions of pre-zygotic eliminations.

I have been doing little of direct genetic consequence so I am at a loss as to just what I should talk about at Oak Ridge. If you intend to go into detail on serotypic transduction in Salmonella I would like to know so that I can plan my presentation. If you are going to solely discuss coli and its ramifications then I would feel free to draw upon the published accounts of serotypic transduction in defining transduction genetically. My present plan is to give a very general operational view of transduction with a bit of speculation upon its genetic aspects. Any comments you might have will be appreciated.

I have been busy since last I wrote with both the mouse salmonellosis and transduction problems.

Mouse salmonellosis and nutrition. With some modification for sensitivity the assay developed last year has been pushed forward such that the isolation of the nutrional entity involved is in sight. I needn't labor you with the details other than to say that fractional procedures including the use of ion exchange resins for final resolution has afforded us with a 10^6 fold concentration of the active material. The major problem is to get find sufficient source material of sufficient activity to make pilot plant production worthwhile. There seem to be vintage years with respect to wheats and this material, as all of our recent sources have been pretty poor as compared with last year. Any how the compound is extremely potent considering the fact that the mouse does the administration by feeding; of the order of 50 ug per day for four days has demonstrable effect. Its mechanism of action will have to await its availability in large amounts.

Transduction.

Incorporation studies

1. The turn-over of FA in each phage cycle is greater than could be explained by dilution.
2. The activity per phage (T/P) rises during the course of phage growth at a rate comparable to that of the growth of phage; thus the total activity goes up as the square of the phage.
3. The activity per phage at any particular burst size varies widely with the physiology of the cells, including the medium of growth and the medium of lysis.
4. Chloromycetin, when used at proper concentrations, will selectively inhibit transduction incorporation while allowing phage growth to proceed once the drug is removed.
5. Attempts to "dirty" phage with FA either invitro or in vivo have failed.

6. The yield of transducing material for one marker is of the order of one per two hundred bacteria with the incorporation continuing to the last increments of phage so that it might possibly be higher if a lysis inhibiting system were available. Since the majority of the bacterial nucleic acid is probably converted to viral nucleic acid I take this to mean transduction is very efficient.

The experiments described above were all accomplished with old lysogenic cells for one particular marker. The assay is not quite linear but sufficiently so that the data can be taken as reliable. I here admit to an unfortunate strategic error, for the problems of assay could have been avoided. The technique is the following; even for virulent phage one uses young sensitive cells but simultaneoulsy infects with a high multiplicity of temperate A- phage and the scoring reverts to the normal for temperate phage grown under similar conditions. In this connection one interesting finding turned up. If one uses young lysogenic cells to assay transduction, no transductions appear until a multiplicity of about five is reached and then rises rapidly. The "lost" transductions can be restored by the addition of about 10 A- particles, showing that this is not an adsorption artifact. This is of course reminiscent of the Boyd effect in lysogenization, so I believe this means that the phage aids in establishing the conditions for transduction as well as lysogenization. It seemed possible that the low multiple infections result in abortive transductions à la trails but not demonstrable in this situation, and that this kind of thing might be responsible for trail formation in general, so I wrote to Bruce to see whether he could explicitly demonstrate the effect in motility transductions. I am now repeating the incorporation studies with a variety of markers differing in initial frequency of transduction and of course using the new assay.

The general picture that emerges is the following. During the course of phage growth there is a fortuitous feed in of transducing material with an increasing probability in time perhaps reflecting degrees of nuclear disintegration. Phage growth is quite independent of this process and thus the F_A does not act as any particular virus gene. If the phage lysis a secondary host the F^A is extraneous to the phages genetic continuity and is lost. If the phage enters an immune system it aids in triggering the "stabilization ? of the material but the two can therafter be separate.

I have as you see given up the notion that phage and transducing particles are different. In this connection there is one interesting phage mutant; it induces an unstable lysogenic condition. The transductions are normal in frequency but are all mottled and segregating the phage and not the transduction.

Am looking forward to seeing you at Oak Ridge. Please give my best to everyone in the lab.

Sincerely,

Norton